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Appendix C

REVIEW ARTICLE

Tumor Necrosis Factor Ligand Superfamily: Involvement in the Pathology of Malignant Lymphomas

By Hans-Jürgen Gruss and Steven K. Dower

PHYSIOLOGIC and pathologic activities of cytokines are mediated by binding to cell surface receptors (R). Sequence analysis of cytokine receptors defines several subfamilies of membrane proteins with specific homology of functional domains. Receptor subfamilies of related proteins form (1) the Ig superfamily (eg, interleukin-1 receptors [IL-1Rs], fibroblast growth factor (FGF) Rs, platelet-derived growth factor (PDGF) Rs, c-kit, c-fms, flt-3) characterized by varying numbers of Ig-like repeats in the extracellular domain; (2) the hematopoietin (cytokine) receptor superfamily (eg, erythropoietin receptor [EPOR], growth hormone receptor [GHR], prolactin (PRL) Rs, mpl, ciliary neutrophic factor (CNTF) Rs, leukemia-inhibitory factor receptor [LIFR] gp130, IL-2R β and γ chain, IL-3R, IL-4R, IL-5R, IL-6R, IL-7R, IL-9R, granulocyte colony-stimulating factor receptor [G-CSFR], and granulocyte-macrophage–CSFR [GM-CSFR]) with conserved cysteine residues and the characteristic WSXWS motif; and (3) the tumor necrosis factor (TNF)/nerve growth factor (NGF) receptor superfamily based on cysteine-rich repeats in the extracellular region.[1-5] Further, receptors can be functionally, based on signaling, subdivided by common receptor subunits involved in forming several multimeric receptor complexes such as the gp130-associated proteins for IL-6R, LIFR, OncoMR, CNTFR, and IL-11R; common β-chain–associated molecules for IL-3R, IL-5R, and GM-CSFR complex; and common γ-chain–associated members (IL-2R, IL-4R, IL-7R, IL-9R, IL-13R, and IL-15R).[6]

The TNF/NGF receptor superfamily contains at present 10 different membrane proteins and several viral open reading frames encoding TNFR-related molecules. The p75 low-affinity NGF receptor was the first cloned receptor of this family.[7] TNF was originally discovered by its antitumor activity in mice.[8] Subsequently, cloning of two specific receptors for TNF showed that there were related to the NGFR.[9-11] In recent years a new type-I–transmembrane TNF/NGF receptor superfamily has been established.[1,2,4,5] The TNF/NGF receptor superfamily includes the p75 NGFR,[7] p60 TNFR-I,[9-11] p80 TNFR-II,[10] TNFR-RP/TNFR-III,[12] CD27,[13] CD30,[14] CD40,[15] 4-1BB,[16] OX40,[17] and FAS/APO-1.[18,19] In addition, several viral open reading frames encoding soluble TNFRs have been identified, such as SFV-T2 in Shope fibroma virus[20] and Va53 or SalF19R in Vaccinia virus.[21,22] These receptor superfamily is characterized by multiple cysteine-rich domains in the extracellular (amino-terminal) domain, which have been shown to be involved in ligand binding.[2,10,23-25] The average homology in the cysteine-rich extracellular region between the human family members are in the range of 25% to 30%.[5,26] The NGFR, TNFR-I, TNFR-II, and FAS/APO-1 have a broad tissue distribution, whereas the others (CD27, CD30, CD40, 4-1BB, and OX40) are mainly restricted to cells of the lymphoid/hematopoietic system.[4]

Ligands for these receptors have been identified and belong to two recently formed cytokine superfamilies. The neurotrophins (NT; NGF ligand superfamily) are basic, NGF-like dimeric soluble molecules and include NGF, BDNF, NT-3, NT-4, and NT-5.[27,28] The ligands of the TNF ligand superfamily are acidic, TNF-like molecules with approximately 20% sequence homology in the extracellular domains (range, 12% to 36%) and exist mainly as membrane-bound forms; the biologically active form is a trimeric/multimeric complex. To this group belong TNF,[29-32] LTα,[33,34] LTβ,[35] CD27L,[36] CD30L,[37] CD40L,[38-42] 4-1BBL,[43,44] OX40L,[45,46] and FASL.[47-50] Soluble forms of the TNF ligand superfamily have only been identified so far for TNF, LTα, and FASL.[29,47,51,52] These proteins are involved in regulation of cell proliferation, activation, and differentiation, including control of cell survival or death by apoptosis or cytotoxicity.[4,5]

Malignant lymphomas are a heterogenous group of lymphoid tumors that mainly arise from the lymphoreticular system and are grouped, based on morphologic criteria, into two large categories, Hodgkin's disease (HD) and non-Hodgkin lymphomas (NHL).[53] Furthermore, lymphomas are grouped into three major phenotype categories: B cell, T cell, and HD. The origin of the lymphoma cells in NHLs is most commonly from B cells (90%) and less frequently from T cells (10%), with massive clonal expansion of the malignant cell population. In contrast, HD is defined by common clinical and pathologic feature.[54-56] The diagnosis of HD is typically based on a disrupted lymph node architecture and the presence of the malignant mononucleated Hodgkin and multinucleated Reed-Sternberg (H-RS) cells embedded in an abundance of normal, reactive cells (eg, lymphocytes, histiocytes, eosinophils, plasma cells, and stromal cells) without malignant transformation.[57] HD contains only a low proportion of the neoplastic H-RS cells, accounting for usually less than 1% to 2% of the total tumor cell mass.[54,58-60] The etiology of HD and the origin of the H-RS cells remains unclear with almost every cell type having been described as a normal counterpart.[61-63] Primary and cultured H-RS cells express a heterogenous panel of cytokines and cytokine receptors that correlate with the typical clinical and pathologic presentation of HD cases.[56,64] Cytokines and a cell contact-dependent activation network are critical elements in the pathology of HD.

This review will summarize recent data on the functional

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role and pathobiologic involvement of the TNF/NGF receptor and TNF ligand superfamilies for the pathology of malignant lymphomas.

STRUCTURAL FEATURES OF TNF RECEPTOR AND LIGAND SUPERFAMILIES

The TNF receptor superfamily consists, at present, of 10 mammalian proteins.[4,10,37,65] In addition, several viral open reading frames (ORFs) have been identified that encode acquired soluble TNF receptors (eg, SFV-T2, va53, SalF 19R, MYX-T2, G4R, and crmB).[10,20-22,66-70] The TNF ligand superfamily contains nine members, ie, the counterparts of the receptors.[5] The ligand for the low-affinity NFG receptor is structurally unrelated to the TNF superfamily of ligands.[27]

The crystal structures of TNFRI (p60), TNF, and LTα have been solved[71-73] and the mechanism of receptor-ligand interaction was illustrated.[73,74] The ligand is a trimer.[71,72] The receptor extracellular ligand binding region is a rod-like structure in which the four cysteine-rich 40 residue repeats each folds tightly together and forms intimate longitudinal contacts with its neighbors. The complex contains one LTα/TNF homotrimer and three receptor chains.[73] The receptors bind in three grooves in the ligand trimer formed by the subunit interfaces; thus each receptor makes contact with two subunits. The structure predicts that the binding of ligand will crosslink three receptors together into a cluster. Recent reports suggest that, for LTα, this model may be an oversimplification. A second form of lymphotoxin (lymphotoxin-β [LTβ] or p33) has been identified[35,75,76] that unlike LTα, appears to be membrane anchored. The TNFR-related protein (TNFRP) has been shown to be a specific receptor for LTβ and hence is TNFRIII.[77] In addition, it appears that LTα and LTβ, when expressed in the same cell, can form heterotrimeric complexes.[76] Because the sites for receptor binding lie at the subunit interfaces, such heterotrimers can contain three different sites (α_2, β_2, and $\alpha\beta$) in various combinations depending on subunit stiochiometry. In which way such heterotrimeric ligands interact with cells expressing combinations of TNFRI, TNFRII, and TNFRIII needs to be clarified.

The overall structures of two TNFR-like viral gene products, the TNF receptors and the ligands, are shown in Fig 1. In addition, Tables 1 and 2 summarize the sizes of the proteins as lengths of the various sequence segments and chromosomal location. Sequence alignments of the extracellular regions of the receptors show that they are distantly related (25% to 30% in general; summarized in Table 3). However when sequence conservation patterns are examined, two features are immediately apparent.[4,10] First, there is a characteristic pattern present in all the sequences, with the majority of conserved positions containing an unusually large number of cysteine residues. Second, all of the proteins are composed of several repeats of a core domain of 30 to 40 residues.[4,10,25] The TNFRI crystal structure shows that this domain is composed of three elongated strands of residues held together by a twisted ladder of three disulphide bonds[73]; the strands that form the core of the structure contain approximately 25 residues, of which 6 are cysteines. The strands are joined by loops of less-conserved structure. Thus, the three-dimensional structure shows that the cysteine-rich repeats in the sequence present the charcteristic structural domain in this family. TNFRI contains four such domains stacked longitudinally to form a bent rod. This rod structure thus forms an extended ligand binding unit, with the ligand contact side chains being located in domains 2 and 3. The largest variation in structure between the family members is found in the region between the C-terminus of the membrane proximal domain and the transmembrane region. This segment varies from 8 residues in FAS[18] to 70 residues in CD27[13] and is presumed to form a variable spacer between the ligand binding unit and the membrane. CD30 has an extracellular region in which the cysteine-rich repeat unit has been duplicated.[14] The observation that the CD30a and CD30b regions are far more closely related (50%; see Table 3) than any of the other family members are, suggests that this structure has arisen from tandem duplication event more recent than those that gave rise to the various family members from a common ancestor. CD30a is as related to the rest of the family as any of the other members, but CD30b, the membrane proximal region, is the most divergent sequence of the family (Table 3). It seems likely therefore that it has evolved rapidly away from the rest of the family, may well have lost ligand binding activity, and serves the purpose of an extended spacer.

It is striking that this family of molecules shows a relatively low level of sequence conservation despite in all likelihood sharing a common fundamental structure. This finding suggests that the sequences have diverged rapidly. In support of this notion, comparisons of the sequences of the same member from different species show unusually low levels of conservation. This finding can be seen from two examples included in Table 3, ie, OX40 and CD40. In both cases the human and murine forms are only approximately 60% identical. For example, by contrast, the murine and human insulin receptor precursors are 95% identical when comparing the entire 1370 residues sequences. The selection pressure driving rapid divergence of the TNF receptor family members may well arise from the subversion of these systems by pathogens. Thus, a number of TNFR viral ORFs (SFV-T2, va53, SalF 19R, MYX-T2, G4R, and crmB) have been detected by sequence homology and found to be soluble TNF binding proteins capable of blocking TNF action.[20,22,69] These viral genes were presumably acquired from a host species by recombination and confer a selective advantage for the viruses by attenuating host immune and inflammatory responses. As a corollary of this argument, one would expect that evolution of a mutated ligand-receptor pair that was no longer inhibited by the viral gene product would be advantageous to the host.

Comparison of the cytoplasmic sequences of the receptors shows these to be considerably more diverse than the extracellular regions. Indeed, these differ markedly not only in sequence but also in size (Tables 2 and 3), and multiple sequence alignments show no evidence of any underlying shared structure running through the family. One striking comparison is that between murine and human CD40; whereas the membrane proximal 34 residues are 78% identical, the murine molecule possesses an additional 27 residues

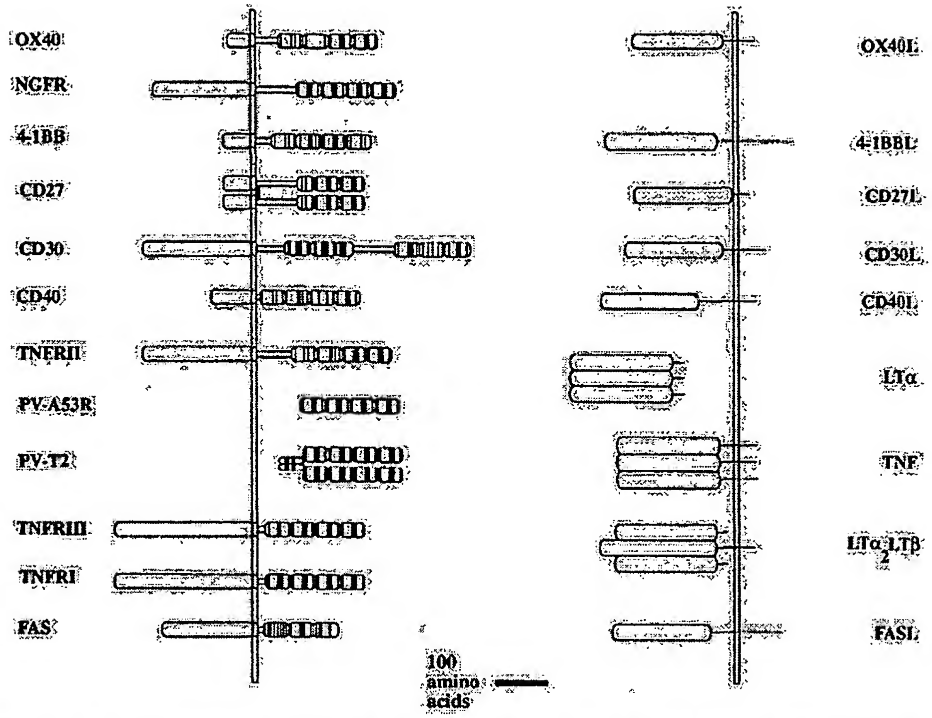

Fig 1. The TNF receptor and ligand superfamilies. Schematic presentation of the 10 members of the TNF receptor superfamily and two examples of viral ORFs (PV-A53R and PV-T2) encoding soluble TNFR homologues (left panel). The TNF receptor family members are characterized by variable numbers of cysteine-rich repeats in the extracellular domain. The homologous domains are shown as sequested open boxes, with the cysteine residues indicated by lines. TNFRIII is identical to the TNFR-RP (related protein). In addition, the nine members of the TNF ligand superfamily are shown in the right panel. The extracellular homologous C-terminal regions are indicated by open boxes and the nonhomologous sequences by lines. LT is shown in both the homotrimeric, secreted LTα form and the heterotrimeric, membrane-anchored $LT\alpha_1LT\beta_2$ complex. Pro-TNF and FASL can be proteolytically cleaved for the release of active soluble forms. NGF is a basic, soluble dimeric molecule and the prototype of the NGF ligand superfamily. NGF has no homology to the TNF ligands and is therefore not included.

at the C-terminus. There are some elements shared between subsets of family members, thus TNFRI and FAS share the so-called "death domain",[78-80] but this sequence element is missing in the other two TNF receptors (TNFRII and TNFRP/TNFRIII).[10,12,77] Whether this degree of diversity is the consequence of rapid accumulations of mutations in this region of the genes for these molecules or of recombination events is not clear. However, it seems reasonable to suppose that there is unlikely to be any common immediate-early signalling event triggered through all these receptors. Indeed comparison of the sequences of this group of molecules as a whole with the databases fails to identify any motifs that resemble other families of signalling molecules, thus offering no clues as to what the immediate-early events for any of these molecules might be. Relatively little general information is yet available about the signal pathways to which the more recently characterized family members (CD40, CD30, 4-1BB, CD27, OX40, and FAS) might couple, but a diverse set of signalling pathways seems likely for the divergent biologic responses. Most data for signalling through TNF receptors have come from studies using the TNFRI (p60) and TNFRII (p80) system and have been reviewed in detail recently.[81]

The extracellular regions of TNF ligand family, like the receptors, are highly diverse in sequence, with identity levels in the region of 20% for different family members. The murine and human forms of the same molecule are somewhat more conserved than the corresponding receptors, being approximately 80% identical (Table 3C). However, sequence alignments show that as with the receptors there is a characteristic pattern of sequence conservation, there being 9 short regions of conserved sequence distributed along the length of the molecules (Fig 2). Superposition of the sequences on the three-dimensional structures of TNF and LTα shows that these regions correspond to the strands that form the core of the protein. The residues that lie on the loops joining the strands show no detectable conservation. Thus the ligand family has diverged as rapidly as the receptors. Several of the ligand superfamily members have moderate sized cytoplasmic regions, and at least some are capable, when engaged by their receptors, of delivering signals (eg, CD27L, CD40L, and 4-1BBL).[82-84] The signal pathway is presently unknown.

CHARACTERISTIC BIOLOGIC PROPERTIES OF TNF LIGAND SUPERFAMILY MEMBERS

TNF and LTα, products of activated macrophages and T cells can kill some transformed cell lines, mediate cell activation and proliferation, and are functionally linked as primary mediators of immune regulation and inflammatory response.[85,86] TNF has a pathogenic involvement, eg, in septic

Table 1. Molecular Characteristics for the Human TNF Receptor and Ligand Superfamily Members

Molecule	Molecular Weight (kD)	Length (amino acids)	Chromosomal Location
TNFR family			
CD27	45-55	242	12p13
CD30	120	578	1p36
CD40	50	258	20q11-13
4.1BB*	35	234	1p36
OX40	50	250	1p36
FAS/APO-1	45	320	10q24.1
TNFRI (p60)	60	435	12p13
TNFRII (p80)	80	440	1p36
TNFRIII (TNFR-RP)	~75	414	12p13
NGFR p75	75	402	17q21-22
TNFL family			
CD27L	50	193	19p13.3
CD30L	26-40	234	9q33
CD40L	33	261	Xq26-27
4.1.BBL*	50	309	19p13.3
OX40L	26-28	183	1q25
FASL	40	281	1q25
TNF	17 (s) and 26 (m)†	233	6 (MHC)‡
LTα	25	205	6 (MHC)
LTβ	33	244	6 (MHC)

* 4-1BB and 4-1BBL represent the murine proteins.

† Soluble (s) and membrane-bound (m) forms of TNF.

‡ TNF, LTα, and LTβ chromosomal mapping as a cluster within the location for the major histocompatibility complex (MHC).

shock, some autoimmune disorders, malignancies, and graft-versus-host disease.[8]

The nine TNF-related cytokines show distinctive but overlapping cellular responses for developmental and regulatory networks involving cells of the lymphoid, hematopoietic, and other lineages, such as stromal cells and neuronal cells.[4,5] At least some of the nine TNF ligand superfamily members (eg, TNF, LTα, LTβ, and CD40L) form trimeric proteins (see above).[35,71,72,87-89] In general, the TNF ligand superfamily members exert their biologic activity by causing receptor multimerization at the cell surface.[73] As mentioned above, the LTα homotrimer is the only entirely secreted protein and the TNF homotrimer is active after proteolytic release form the cell surface.[29,51,52] The biologically relevant forms of the other family members are membrane-bound type II glycoproteins.[35-38,43,45-47,90] Natural soluble forms for CD27L, CD30L, CD40L, 4-1BBL, and OX40L have not been reported. The exception is FASL, which exists in the predicted membrane-bound form but also as a soluble shed form in COS cell supernatants with presently unknown biologic relevance.[47]

Most of the TNF receptor superfamily members exist also in a soluble form, released by proteolytic cleveage (eg, TNFR p60, TNFRp80, CD27, CD30, CD40, and FAS) or through alternative splicing (eg, 4-1BB).[43,91-103] Although the cytoplasmic domains of most TNF receptor superfamily members are divergent from each other, several biologic functions, such as cytotoxic signals, induction of proliferation and differentiation, and cellular activation, are shared between two or more ligands.[5] Biologic activities related to T-cell–mediated immunity are a unique feature for all members of the TNF ligand superfamily.[8,35-37,43,45,47,104] All ligands and receptors, without exception, are expressed on activated T cells (Table 4). Purified human T cells and T-cell clones show enhanced proliferation when stimulated with any recombinant TNF family ligand or crosslinked with antireceptor antibodies in the presence of anti-CD3 or other mitogens, such as phytohemagglutinin (PHA), phorbol myristate acetate (PMA), or ionomycin.[8,35-37,43,45,47,104] Possible autocrine T-cell activation and growth control might be a common feature of this protein family. The induction of each ligand expression shows unique kinetics consistent with different roles for each of these ligands in the T-cell activation.[105] For example, the induction of CD30L surface expression on activated T cells is slower in comparison to other TNF ligands such as TNF, CD27L, CD40L, and 4-1BBL (maximal expression, 24 hours *v* 6 hours, respectively). B-cell proliferation and Ig secretion is induced by at least TNF, LTα, and CD40L. Further, several members participate in T-cell–dependent help for B cells, which are known to express TNFR-I, TNFR-II, CD27, CD30, CD40, FAS, and 4-1BB (Table 4).[106,107] TNF, LTα, and CD40L are mitogenic to B cells.[38,108-110] TNF, CD30L, and 4-1BBL are also abundantly expressed by activated macrophages.[30,37,43,84] Signals generated by TNF superfamily ligands in target cells are productively coordinated with accessory molecule expression (eg, LFA-1, ICAM-1, and B7 ligands).[111,112] For example, TNF, LTα, CD30L, and CD40L are capable of inducing cellular aggregation and upregulation of LFA-1 (CD11a)/ICAM-1

Table 2. Structural Characteristics of TNF Receptor and Ligand Superfamily

	No. of Amino Acid Residues			
	Extracellular	Transmembrane	Cytoplasmic	Extracellular Domains
Receptors				
CD27	175	21	46	2.5
CD30	360	27	211	5.5
CD40	175	21	62	4
FAS	156	20	144	3
OX40	188	26	36	3.5
TNFRI	190	25	220	4
TNFRII	240	27	173	4
TNFRIII	201	26	187	4
4-1BB	159	30	45	3.5
NGFR	225	23	154	4
Ligands				
CD27L	165	16	12	
CD30L	172	26	36	
CD40L	216	23	22	
LTα	170	24	11	
LTβ	197	31	16	
OX40L	139	21	23	
TNF	176	28	29	
4-1BBL	206	21	82	
FASL	179	27	75	

With the exception of 4-1BB and 4-1BBL, all sequences used in the analysis were those of human proteins.

Table 3. Sequence Relationships Between Members of the TNF Receptor Superfamily and Between Members of the TNF Ligand Superfamily

A. Receptor Extracellular Regions

	MuOX40	HuNGFR	Mu4-1BB	HuCD27	HuCD30a	HuCD30b	HuCD40	MuCD40	HuTRII	HuTR-RP	HuTRI	HuFAS
HuOX40	63.8	37.5	27.0	28.0	24.2	20.7	33.0	30.2	35.3	28.0	20.9	26.2
MuOX40	—	31.6	26.3	29.3	26.5	18.9	32.0	30.3	34.3	32.1	26.8	26.7
HuNGFR	—	—	25.3	25.8	25.6	25.8	25.6	28.4	26.4	26.3	34.4	23.3
Mu4-1BB	—	—	—	26.4	16.8	16.9	30.8	32.6	31.7	29.5	17.3	27.5
HuCD27	—	—	—	—	19.5	22.5	22.8	25.7	25.5	23.3	21.3	25.7
HuCD30a	—	—	—	—	—	50.9	24.2	25.2	28.7	29.3	29.1	23.1
HuCD30b	—	—	—	—	—	—	19.1	21.0	24.4	18.6	18.7	16.0
HuCD40	—	—	—	—	—	—	—	59.3	37.0	35.3	26.1	31.1
MuCD40	—	—	—	—	—	—	—	—	32.7	30.1	27.2	35.3
HuTNFRII	—	—	—	—	—	—	—	—	—	32.0	23.3	25.2
HuTNFR-RP	—	—	—	—	—	—	—	—	—	—	27.0	33.8
HuTNFRI	—	—	—	—	—	—	—	—	—	—	—	28.9

B. Receptor Cytoplasmic Regions

	MuOX40	HuNGFR	Mu4-1BB	HuCD27	HuCD30	HuCD40	MuCD40	HuTRII	HuTR-RP	HuTRI	HuFAS
HuOX40	61.1	22.2	18.9	13.9	30.5	16.1	21.6	18.3	27.0	14.3	17.4
MuOX40	—	24.2	26.7	23.3	11.4	14.3	14.3	11.7	33.3	30.3	8.8
HuNGFR	—	—	18.2	19.6	18.8	27.6	16.9	21.4	15.5	20.7	15.2
Mu4-1BB	—	—	—	28.5	19.5	21.6	19.0	17.1	24.3	12.2	7.5
HuCD27	—	—	—	—	28.9	18.6	23.4	11.9	20.9	21.7	18.6
HuCD30	—	—	—	—	—	33.8	15.6	24.7	27.2	18.6	9.7
HuCD40	—	—	—	—	—	—	77.9	23.3	23.7	21.0	25.0
MuCD40	—	—	—	—	—	—	—	20.8	20.7	15.9	11.4
HuTNFRII	—	—	—	—	—	—	—	—	20.1	16.1	15.6
HuTNFR-RP	—	—	—	—	—	—	—	—	—	21.4	14.5
HuTNFRI	—	—	—	—	—	—	—	—	—	—	23.9

C. Ligand Extracellular Regions

	Mu4-1BBL	HuCD27L	HuCD30L	HuCD40L	MuCD40L	HuTNF	HuLTα	HuLTβ	HuFASL	MuFASL
HuOX40L	13.6	17.7	20.9	20.3	19.3	15.5	20.0	17.8	23.5	22.1
Mu4-1BBL	—	22.6	23.8	20.1	20.2	23.2	19.9	23.4	23.1	24.5
HuCD27L	—	—	20.7	27.9	20.9	23.1	22.1	22.1	21.4	20.3
HuCD30L	—	—	—	23.0	21.3	25.5	16.3	20.4	22.1	24.7
HuCD40L	—	—	—	—	75.2	26.0	21.7	24.4	24.9	24.6
MuCD40L	—	—	—	—	—	30.1	21.7	23.1	23.7	24.0
HuTNF	—	—	—	—	—	—	33.5	26.0	26.4	31.7
HuLTα	—	—	—	—	—	—	—	30.9	29.6	28.2
HuLTβ	—	—	—	—	—	—	—	—	26.4	31.7
HuFASL	—	—	—	—	—	—	—	—	—	80.7

The sequences were compared in individual pairs using BESTFIT. For the receptors, the regions compared were those defined in Table 1. For the ligands, the regions compared were the entire region C-terminal to the membrane spanning sequence. The values given are percent amino acid identity, allowing gaps to be placed to optimize the fit. The extracellular region of CD30 contains the same concensus sequences as the other receptors, but has been duplicated (see Fig 1) to be able to compare it directly with the other members; each of the duplicated regions was treated separately. CD30a is residues 23-190 of CD30 (numbered from the initiator Met) and CD30b is 191-379.

Abbreviations: Hu, human; Mu, murine; TRII, TNFR II (p80); TRI, TNFR I (p60); TR-RP, TNFR-RP (related protein).

(CD54) expression.[113-117] CD30L and CD40L share the ability to induce B7-1 and B7-2 expression, part of the strongest known T-cell costimulatory pathway.[117-120] In general, all TNF ligand superfamily members, including FASL and CD40L, are essential for T-cell costimulation and activation. It is of special interest that signals, at least through CD27L, CD40L, and 4-1BBL, can provide costimulation for activated peripheral blood (PB) T cells.[82-84] Further studies need to be performed to see if other TNF ligand superfamily members are able to transduce a costimulatory signal.

The ability to induce cell death (necrosis and/or apoptosis) is another unique feature of this family and is presently established for TNF, LTα, CD30L, 4-1BBL, and FASL.[30,33,37,44,47,121,122] FAS and TNFRs expression is found broadly on both myeloid, lymphoid, and stromal cells.[10,123-125] FAS monoclonal antibodies (MoAbs) and FASL induce apoptotic (programmed) cell death and FAS/FASL interaction appears to be involved in T-cell repertoire formation, including positive or negative selection, suggesting a role of the FAS-FASL interaction in peripheral T-cell tolerance.[47,126-136] Interestingly, the cytoplasmic domains of the 60-kD TNFR and the FAS antigen contains a 65 amino acid "death domain," which is critical for signal transduction of the cytotoxic effects.[78,137] Both receptors still use at least partially distinct signaling path-

ways involved in apoptosis.[80] The cytoplasmic domains of the p60 and p80 TNFRs are unrelated and the signaling of the p80 TNFR for cell death and in mediating TNF responses in general remains controversial.[78,79,93,138-147]

Essential roles of several members of the TNF receptor or ligand family have been confirmed by naturally occuring or induced mutants that abolish the functional expression of the individual receptor/ligand protein. Naturally occuring inactivating mutations of the FAS antigen (lpr mouse) and the FASL (gld mouse) cause both similar lymphoproliferative diseases with lymphadenopathy and autoimmune disease, suggesting a failure of the immune system to eliminate autoreactive T cells.[49,50,148] CD40 and CD40L knock-out mice confirm that mutations of CD40L cause X-linked immunodeficiency, with high levels of IgM and low levels of IgG (block for Ig isotype switching).[149-151] Hyper-IgM patients show normal numbers and biologic function of B cells, but failure of T-cell–dependent B cell help because of nonfunctional CD40L.[39,152-155] Experimental deletion of the 60-kD TNFR gene in mice causes immunodeficiency with severely impaired clearance of bacterial pathogens and rapid death caused by infection, but resistance to the lethal effect of lipopolysaccharides (LPS).[156,157] Lack of 80-kD TNFR showed only a minimal phenotype with modest resistance to the lethal effect of TNF. In addition, functional ablation of TNF and LTα by overexpression of a neutralizing TNF inhibitory fusion protein (60-kD TNFR extracellular domain fused to mouse IgG heavy chain) in mice show pronounced LPS and TNF resistance with comparable phenotypic effect seen for the homozygous deletion of the 60-kD TNFR gene.[158,159] Furthermore, the deletion of the LTα gene results in a distinctive phenotype, characterized by the absence of structured lymph nodes and disordered splenic architecture.[160] In summary, several members of the TNF ligand and receptor superfamilies play crucial roles for lymphoid and thymic development, T-cell–mediated immune responses, T-cell–dependent help for B cells, and humoral B-cell activity. The detailed interactive network for the immune response and lymphoid differentiation mediated by the TNF-like ligands needs further evaluation.

CD27L AND CD70 ARE IDENTICAL MOLECULES AND ARE EXPRESSED ON DIFFERENT LYMPHOMAS

The CD70 antigen was originally identified by the Ki-24 MoAb.[161] CD70 is expressed on many peripheral T- and B-cell lymphomas (50% to 70% of cases positive) with frequent CD70 positivity observed within the cytoplasm.[162] The strongest expression is found on H-RS cells of HD (96% to 100% of cases positive) followed by large-cell NHLs (60% to 80% of cases positive). CD70 expression was not found on lymphoma cells derived from precursor T and B cells, such as lymphoblastic lymphoma or acute lymphoblastic leukemia (ALL). Permanent cell lines showed high CD70 expression only in those cell lines related to activated cells (eg, antigens, mitogens, and viral-transformed cells), but not in those resembling precursor T or B cells. In most cases, expression of the CD70 molecule is associated with the expression of other activation antigens, particularly CD25 and CD30.[162] However, 20% of B-cell NHLs and 5% of T-cell NHLs expressed only CD70 antigen on the lymphoma cells.[162]

For the immune system, CD70 is absent from resting lymphocytes, but can be induced after activation.[162] CD70 antigen is detectable on PHA-stimulated T and B lymphocytes after 24 hours and peaked at 96 hours, with 70% of stimulated peripheral blood B cells and 25% of T cells expressing the antigen.[162] CD70 expression could not be detected on resting or IFN-γ–treated monocytes, neutrophils, or dendritic cells. Recently, the CD70 gene was cloned and found to be identical to the cloned ligand for CD27 (CD27L).[36,83,90] CD27 is expressed by medullary thymocytes, most peripheral blood T cells, a subset of mature B cells, and NK cells.[163-170] CD27 expression on T cells is associated with the helper phenotype (naive T cells with CD45RA$^+$), whereas most memory T cells (CD45RA$^-$,CD45RO$^+$) lack CD27.[98,165,171] Activation of T cells results in upregulation of CD27 expression as cell surface molecule but also in the release of a soluble 28- to 32-kD form of CD27 (sCD27).[98-101,165,171] The proteolytic shed sCD27 molecule can serve as a marker for the immune activation in vivo.[13,172] The distribution and regulation pattern of CD27 for T cells supports a CD27 function for more naive/unprimed T cells than completely differentiated effector T cells.[98,165,171]

The biologic functions of CD27L include a costimulatory signal for T-cell proliferation, generation of cytotoxic T cells, and enhanced cytokine secretion,[36] but its functional relevance for thymocytes and B cells remains to be elucidated. It is of interest that only CD27$^+$ B cells can be induced to secrete Ig in vitro after stimulation with mitogens or CD27L.[164,165,172] In addition, CD27L antibodies or sCD27 block allogenic B-cell–mediated stimulation of T-cell proliferation.[90,164,165,172,173] Immunohistologic studies show that the CD27L/CD70 molecule is expressed on most lymphocytes in occassional tonsil germinal centers, low number of lymphocytes in the paracortical areas of tonsils, lymphoblasts in the skin and gut, and thymic epithelial cells, but not on cortical and medullary thymocytes.[162,171] Preliminary functional data suggest that the CD27-CD27L/CD70 interaction is part of the network involved in regulation of T-cell activation during antigen-specific immune responses, generation of memory T-cell populations, and expansion of cytotoxic T cells.[174]

A relatively high percentage of T- and B-cell NHLs but also HD express CD27L/CD70; this expression is characteristically high on H-RS cells.[162,175] In addition, most HD-derived cell lines express CD27L surface molecules but not the counterstructure CD27[63] (H.J.G., unpublished observation). B-cell NHL cell lines can express both the CD27 and CD27L/CD70 molecule, but its functional relevance for the growth control of these cells has not been established. CD27 expression was found in 50% of B-cell leukemias and 71% of B-cell NHLs.[176] CD27 was present on malignant B cells corresponding to early stages of antigen-independent B-cell maturation. Pro-B–cell ALLs were CD27$^-$, but 30% of pre-B–cell ALLs were positive. Mature B-cell ALLs had a high level of expression of CD27 and chronic lymphocytic leukemia (CLL), prolymphocytic leukemias, and some hairy cell leukemias (HCL) were moderate to strong CD27$^+$. In addi-

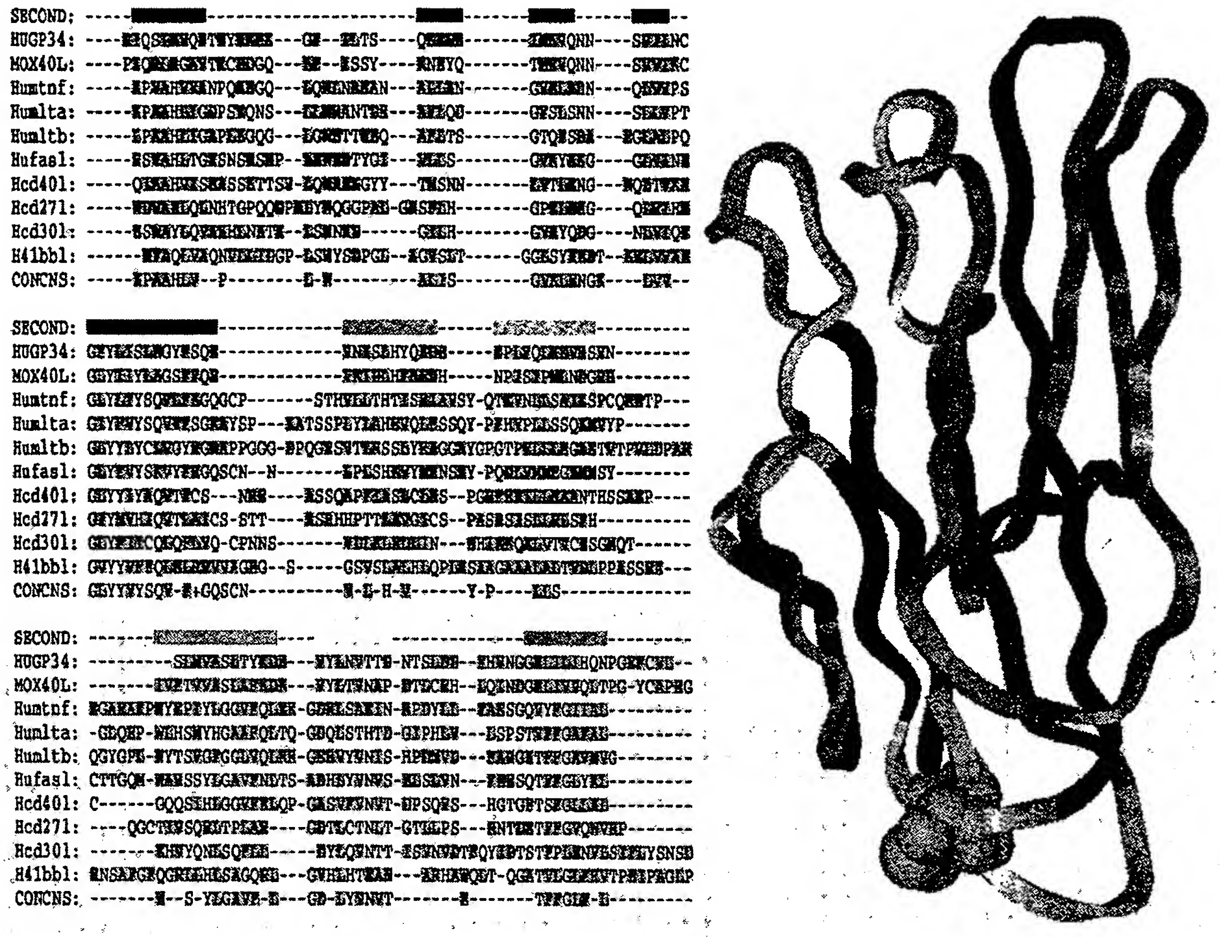

Fig 2. TNF ligand superfamily alignment and predicted tertiary structure based on TNF. Sequence homology is restricted to the C-terminal region of TNF family members (left panel). Colored bars on the top line (SECOND) indicate β-strands of TNF tertiary structure. Conserved amino acid motifs are shown by the line CONCNS. Amino acids are color-coded: green, hydrophobic; blue, basic; red, acidic; yellow, cysteine. (Right panel) Crystal structure of TNF with β-strands colored according to the corresponding sequence homology. Gray strands indicate nonhomologous amino acid. Hu and H, human; m, murine; l, ligand; tnf, tumor necrosis factor; lt, lymphotoxin; cd, CD (cluster designation).

tion, most low-grade diffuse and follicular lymphomas (85% of cases positive) and intermediate- and high-grade lymphomas (62% of cases positive with variable expression levels) expressed CD27 on the malignant B-cell population. Myeloma cells lacked expression of CD27. Furthermore, sCD27 was elevated in the serum of patients with B-cell malignancies and the highest levels were observed in patients with CLL and low-grade NHLs. The sCD27 serum levels showed a strong correlation to the tumor load, indicating sCD27 serum levels as a useful disease marker in patients with B-cell malignancies.[176]

Taken together, CD27 and CD27L/CD70 seem to be expressed with high frequency by the malignant cells of different entities of lymphomas and may serve as markers for tumor burden and disease activity, but any functional correlation to defined pathophysiologic presentation has not been established (Fig 3).

CD30 AN HD-ASSOCIATED ANTIGEN AND ITS LIGAND

HD-derived cell lines were used to develop MoAbs that could be used to visualize H-RS cells in tissue sections. Ki-1, the first CD30 MoAb, was raised against the HD-derived cell line L-428 and described to react uniquely with primary and cultured H-RS cells.[177] A small lymphoid cell population in reactive tonsils was also stained and postulated to be the precursor cells for H-RS cells.[178] Subsequent studies clearly showed that the CD30 MoAb (Ki-1) was neither cell-lineage restricted nor specific for H-RS cells.[179] Over the years, multiple MoAbs against the CD30 antigen (eg, Ber-H2, HeFi, HRS-1, HRS-2, HRS-3, M44, M67, and C10) have been generated with better immunochemical properties than Ki-1.[37,122,180-183] CD30 MoAbs detect a phosphorylated 120-kD membrane glycoprotein and its nonphosphorylated 84-kD precursor protein.[184,185] The cloning of the CD30 antigen has suggested that it might act as a cytokine receptor.[14,186]

In addition to the CD30 staining for a small cell population in the parafollicular area of hyperplastic lymph nodes and tonsils, most blasts appearing during infectious mononucleosis are positive for CD30 expression.[178,181,187,188] CD30 expression has also been detected on a subset of mitogen- or antigen-activated PBTs, Epstein-barr virus (EBV)-

Table 4. Role of the TNF Ligand Superfamily Members for T- and B-Cell Activation Involved in the Immune Response

Function	CD27L	CD30L	CD40L	4-1BBL	OX40L	FASL
Expressing cells	T*, B*, Mf	T*, Mf*, G	T*	T*, S, Mf* B*†	T*†	T*
Responding cells	T*‡, B*, NK	T*, B†, NK	B, EP, Mf*, T*	T*, Mf	T*	T†, B, Mf, G, S
Signaling through ligand	+	?	+	+	?	?
T-cell costimulation	+	+	+	+	+	+
B-cell proliferation		±	+			
Enhanced cytokine secretion	+	+	+		+	+
Ig secretion		−	+			
Upregulation of cell surface antigen expression	+	+	+			+
Upregulation of costimulatory molecules	+	+	+			+
Aggregation/adhesion		+	+			+
Apoptosis/necrosis/AICD		+§	−∥	+		+

Abbreviations: B, B cells; BA, basophils; EN, endothelial cells; EP, epithelial cells; G, granulocytes; M, mast cells; Mf, monocytes/macrophages; NK, natural killer cells; S, stromal cells/fibroblasts; T, T cells; AICD, activation-induced cell death.

*Strong expression after activation.

†Induction of expression after viral transformation (eg, HTLV-1, HTLV-2, EBV, and HIV).

‡CD27 expression is found mainly for naive > memory T cells, but FAS expression for memory > naive T-cell populatons.

§Cytolytic and cytostatic effect on LCAL-derived tumor cell lines.

∥Rescue of germinal center B cells from undergoing spontaneous apoptosis.

transformed B cells, and human T-lymphotropic virus types I and II (HTLV-I and -II) infected lymphocytes or cell lines.[178,180-182,184,189,190] In addition, it has been shown that, among T-cell clones, CD30 is mainly expressed and released as sCD30 from $CD4^+$ and $CD8^+$ T-cell clones producing T_H-2 cytokines such as IL-4 and IL-5.[191-193] Activated tonsil B cells have been reported to be $CD30^+$.[178,181] Similarly, human NK cell clones express the CD30 surface protein.[194] Furthermore, the expression of CD30 was seen by late-stage differentiated PB macrophages.[195] These data are controversial because other groups could not detect CD30 protein or mRNA expression by activated monocytes or macrophages.[63,122,178,181]

CD30 protein and mRNA expression are found in all HD-derived cell lines, with the exception of the myelomonocytic cell line HD-MyZ and cell line SUP-HD1.[14,62,63,122,196] The CD30 antigen is seen on the majority of H-RS cells of most HD cases, with the exception of the lymphocyte-predominant (LP) subform.[61,63] Overall, 84% to 91% of the lymphocyte-depleted (LD), mixed cellularity (MC), and nodular sclerosing (NS) HD cases, but only 32% of LP HD cases express CD30 on the diagnostic primary H-RS cells.[177,178,181,197-199] CD30 expression is not restricted to the malignant cells of HD because CD30 MoAbs also identify a new entity of NHLs with anaplastic morphology ($CD30^+$ anaplastic large-cell lymphomas [ALCL]).[178] In addition to H-RS cells of HD and the subgroup of $CD30^+$ ALCLs, CD30 is also expressed to variable extents on several histologic subtypes of other NHL, such as cutaneous T-cell lymphoma, nodular small cleaved-cell lymphoma, lymphocytic lymphoma, peripheral T-cell lymphoma, Lennert's lymphoma, immunoblastic lymphoma, adult T-cell leukemia/lymphoma

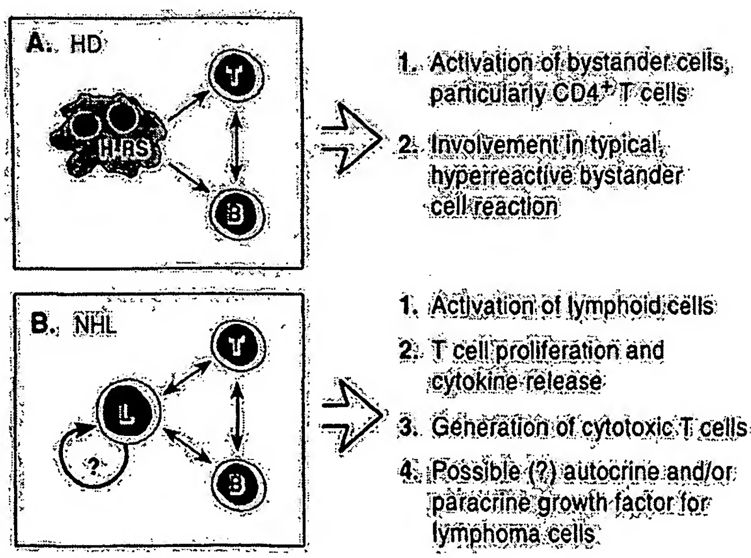

Fig 3. Expression and potential functional involvement of CD27L for HD and NHL. (A) For HD, CD27L (CD70) expression is found for most H-RS cells and surrounding B and T cells. CD27L might be critically involved in hyperreactive bystander cell reaction. (B) NHL cases show frequent coexpression of CD27 and CD27L. CD27L could be an autocrine and/or paracrine growth factor for lymphoma cells.

(ATLL), T-acute lymphoblastic leukemia (T-ALL), and centroblastic/centrocytic follicular lymphoma.[178,197,200-210] Taken together, around 10% of NHLs are positive for CD30 on their malignant lymphoma cells (32% of T-cell NHLs and 4% of B-cell NHLs).[63,186] CD30 expression in T-cell NHLs of different subtpes is restricted to the large-cell variants.[186] The functional relevance of CD30 expression for most entities of NHLs remains presently unclear. The association between CD30 expression and lymphoid malignancies has proven to be a useful pathologic and clinical marker for the identification of malignant cells within lymphoid tissues, particularly lymph nodes. However, expression of CD30 has also been reported on some embryonal carcinomas, nonembryonal carcinomas, malignant melanomas, mesenchymal tumors, some myeloid cell lines, and decidual cells.[181,198,211-213] The CD30 antigen is suitable for immuno-imaging using immunoscintigraphy with radioiodine-labeled antibodies[182] and immunotherapy using immunotoxins (ricin-A or saporin conjugated MoAbs) in HD patients.[186,214,215]

The 85-kD sCD30 molecule is detectable in the serum under restricted conditions.[92] Detectable high sCD30 serum levels were found in the majority of HD cases at diagnosis, more often in patients with advanced disease, bulky tumor, and/or presence of constitutional B symptoms.[216-219] Elevated sCD30 serum levels correlate with the clinical presentation, such as stage, presence of B symptoms, and tumor burden. The sCD30 in the serum of HD patients derives most likely from the $CD30^+$ H-RS cells and is associated with the extent of neoplastic infiltration in HD-involved areas.[177,178,217,218] Most cases with LD HD had positive serum levels, but LP HD patients were frequently $sCD30^-$.[220,221] Soluble CD30 was not detected in any HD patients in complete remission. The levels of sCD30 represent an independent prognostic factor with high sCD30 levels being associated with reduced disease-free survival.[219] The detection of sCD30 seems to be a useful "tumor marker" for $CD30^+$ HD patients based on its correlation to disease activity and presence of H-RS cells.

Recently, the ligand for the CD30 antigen has been cloned and biologically characterized.[37] The presence of the CD30 counterstructure (CD30L) confirms the presumed cytokine receptor function of CD30. The CD30L is a 26- to 40-kD type II membrane glycoprotein, mainly expressed on activated T cells and monocytes/macrophages but also on granulocytes and some Burkitt's lymphoma cell lines.[37,122,222]

Nonpathologic, CD30 expression is largely restricted to antigen-activated T cells and is not detectable before complete and functional antigen receptor rearrangements.[105,178,180,181,184,189,190] IL-2 is further able to enhance mitogen- or antigen-induced CD30 expression on $CD45RO^+$ PBT cells.[190] In contrast, CD30L expression is broader and has been found on $CD4^+$ and $CD8^+$ activated T cells with all CD45 isoforms.[37,105] CD30L costimulates T-cell proliferation[37] and enhances induction of activation antigens, such as ICAM-1, on activated T cells.[105] In addition, CD30L upregulates cytokine secretion of activated PBTs such as IL-2, IFN-γ, and TNF but not IL-4.[105] $CD30^+$ T-cell clones ($CD4^+$ and $CD8^+$) produce preferentially T_H-2 cytokines (eg, IL-4 and IL-5), but peripheral blood T cells stimulated through CD30 release mainly T_H-1 cytokines (eg, IL-2 and IFN-γ).[105,191,192,223] Further studies are needed to determine whether the heterogenous population of $CD30^+$ T cells contains T cells that are capable of developing into both T_H-1 and T_H-2 phenotypes or whether clonal $CD30^+$ T cells are exclusively from the T_H-2 subtype. In general, CD30L is a part of the cascade involved in antigen-induced T-cell activation and proliferation and might play a pathogenic role in several immunologic diseases associated with T_H-2 cytokine pattern (eg, systemic lupus erythematosus, atopic disorders, Omenn's syndrome, and human immunodeficiency virus [HIV] infection).[193]

The analysis of 105 continous human leukemia-lymphoma cell lines for CD30L showed a restricted expression pattern for 6 of the 26 B-cell–lineage tumor cell lines (4 $CD30^-$ Burkitt lymphoma [BL] cell lines, 1 $CD30^-$ BL-like ALL, and 1 NHL).[222] All HD-derived cell lines were CD30L mRNA and surface protein expression negative.[122] Recombinant CD30L was mitogenic for the "T-cell-like" HD-derived cell lines HDLM-2 and L-540 and the T-ALL cell line KE-37 but not for the "B-cell-like" HD-derived cell lines KM-H2 and L-428 or $CD30^+$ BL cell lines.[122] In addition CD30L is capable of enhancing IL-6, TNF, and LTα secretion for HD-derived cell lines and upregulates surface expression of ICAM-1 and B7 family members in a similar fashion as seen for CD40L (see below).[117] CD30L seems to be another paracrine-acting molecule involved in the deregulated cytokine and activation cascade, characteristic for HD.[64] CD30L could be produced by H-RS cell surrounding bystander cells, such as activated $CD4^+$ T cells and activated macrophages and granulocytes (Fig 4). CD30L could modify cytokine secretion of H-RS cells and enhance proliferation and activation of H-RS cells, including upregulation of cell contact-dependent signals. The overexpression of the cytokine receptor CD30 on most H-RS cells appears to be an important clinical, biologic, and pathologic marker for HD (Fig 4). Further understanding of the CD30-CD30L interaction for the oncogenesis of HD will be hopefully generated from studies analyzing CD30L expression in primary HD cases and in vivo models, such as the HD-SCID mice system.

CD30L TRANSDUCES ANTIPROLIFERATIVE SIGNALS TO $CD30^+$ ALCLs

$CD30^+$ ALCLs (approximately 10% of all NHLs) express characteristically high amounts of CD30 on the surface of their clustered malignant lymphoma cells with either T, B, or O phenotype.[178] $CD30^+$ ALCLs are characterized by the presence of large, pleomorphic tumor cells; expression of lymphocyte activation antigens (eg, CD25, CD30, CD71, and MHC class II); a frequent nonrandom chromosomal abnormality (t:2;5); and frequent extranodal disease affecting the skin, lung, gastrointestinal tract, soft tissue, and bone.[178,224-230] $CD30^+$ ALCL can be confused with HD because of the presence of occasional H-RS–like cells and the overlapping immunophenotype of the tumor cells.[178,199,224-227,231] In practice, H-RS cells in HD are frequently $CD15^+$, $CD30^+$, $CD45^-$, EMA^-, in contrast to ALCL tumor cells with $CD15^-$, $CD30^+$, $CD45^+$, EMA^+ immunophenotype.[232,233] HD is in 50% to 60% of cases associated with EBV.[63] The association of EBV with $CD30^+$ ALCLs remains controver-

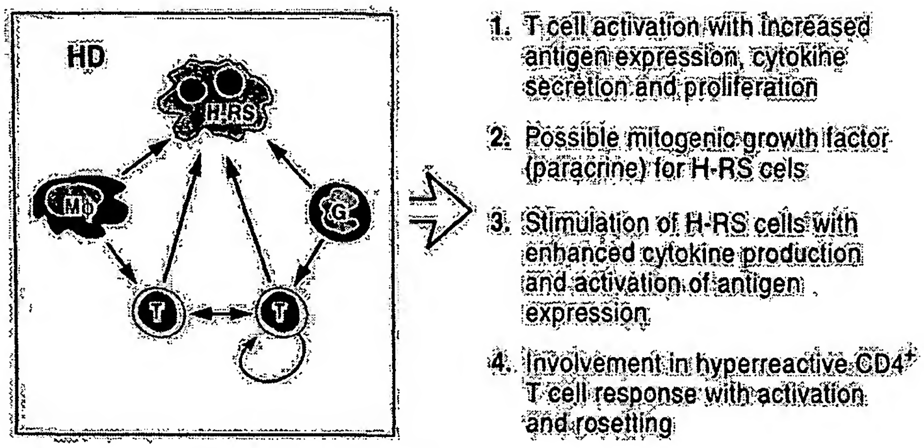

Fig 4. Schematic presentation for the possible functional role of CD30L for the pathology of HD. CD30L expression is found as membrane-bound protein on activated T cells, monocytes/macrophages, and granulocytes (cell types all involved in hyperreactive bystander cell reaction). H-RS cells are CD30L⁻; CD30, as an HD-associated antigen, is expressed by most primary H-RS cells (lymphocyte-predominant subform is an exception). The CD30-CD30L interaction between CD30⁺ H-RS cells and CD30L⁺ bystander cells could be part of the deregulated cell-cell interaction, including growth stimulation, upregulation of activation antigens, and enhancement of cytokine release.

sial (range, 0% to 67% of cases positive), but most data would support the lack of a strong relationship between CD30⁺ ALCLs and EBV.[234-237] These findings suggest that the presence of CD30 is not a simple relation to EBV infection in malignant lymphomas.

Several EBV⁻ ALCL-derived cell lines show strong CD30 expression and could be biologic targets for CD30L. In contrast to the HD-derived cell lines, CD30 mediates reduction of proliferation and cell growth arrest (antiproliferative effect) for most of the CD30⁺ ALCL cell lines.[122] The antiproliferative effect of CD30L includes a cytolytic effect and a cytostatic component with cell cycle arrest.[122] The mechanism for the cytolytic effect is presently unlear, but seems to be FAS-independent and not associated with apoptotic DNA fragmentation. Further investigations have to clarify the interaction between tumor growth of the CD30⁺ anaplastic lymphoma cells, tumor progression/regression, and CD30L expression in ALCL-involved tissues (Fig 5).

It is of interest that the t(2;5) translocation is a common chromosomal finding for CD30⁺ ALCLs.[228,230] This rearrangement fuses the nucleolar phosphoprotein nucleophosmin (NPM) gene on chromosome 5q35 to the novel tyrosine kinase gene ALK (anaplastic lymphoma kinase) on chromosome 2p23.[238] The association of the overexpression of the truncated ALK-fusion protein with the malignant transformation in the CD30⁺ ALCLs is presently unclear, but an 80-kD protein tyrosine kinase (identical to ALK) has been suggested to physically interact with CD30.[239] It is of interest that only a fraction of CD30⁺ ALCL (12% to 40%) cases show the rearrangement of the NPM gene and that at least

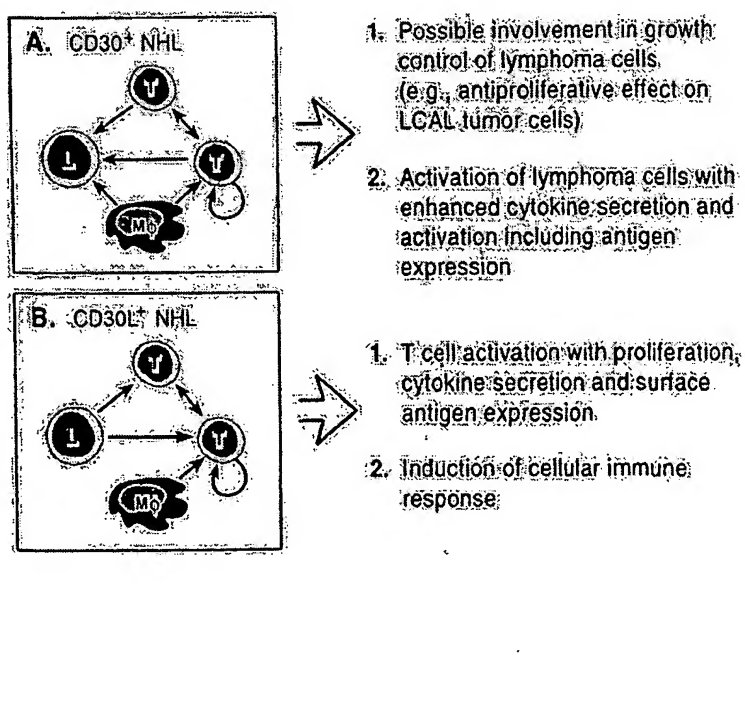

Fig 5. Pathologic role of CD30 and CD30L for NHLs. NHL cells (L) express either CD30 (A) or CD30L (B). CD30 expression of NHL cells could be involved in cellular activation, such as release of cytokines or enhanced expression of activation antigens, and in growth control. A subgroup of ALCL cells use CD30 to mediate an antiproliferative effect. The main source of CD30⁺ cells is activated T cells. CD30L⁺ lymphoma cells might induce T-cell activation and cellular immune responses.

a fraction of HD cases may contain the NPM/ALK fusion transcripts (3 studies using reverse transcriptase-polymerase chain reaction [RT-PCR]: 0 of 40, 2 of 9, and 9 of 13 HD cases rearranged).[240-242] Further studies have to show whether HD cases with rearranged NPM gene represent ALCLs that mimic HD or whether it presents a separate HD subform closely related to ALCL. In addition, CD30$^+$ ALCLs present frequently with abnormal c-myc gene products (6 of 18 cases [33%]), but the functional significance of this is unclear.[243] Taken together, recently several molecular alterations for CD30$^+$ ALCLs have been identified. Further studies will hopefully connect these molecular abnormalities with the functional role of CD30-CD30L interaction for oncogenesis and pathogenesis of ALCLs. In addition, the prognostic, biologic, and functional role of CD30 expression of the malignant cells of other NHLs needs further evaluation.

CD40L SHARES COMMON BIOLOGIC ACTIVITIES WITH CD30L FOR HD

CD40 is a 50-kD glycoprotein and is expressed on a variety of cell types, including normal, virally transformed and malignant B cells (see below for more details), but also monocytes, activated T cells, follicular dentritic cells, interdigitating reticulum cells, thymic epithelium, and some epithelial carcinomas.[15,162,244-246] Recently, the murine and human CD40L have been characterized and cloned.[38-42] The CD40L is a 33- to 39-kD type II membrane glycoprotein and is expressed primarily on the surface of activated CD4$^+$ T cells but also on some CD8$^+$ T cells, mast and stromal cell lines, and basophils.[40,247,248] Studies using MoAbs to CD40 or recombinant CD40L have shown diverse biologic activities as a result of signaling through CD40.[65,249] These activities include the proliferation of B cells and induction of Ig secretion in the presence of other cytokines.[250] Furthermore, CD40L-CD40 interactions mediate rescue of germinal center centrocytes from apoptosis.[251,252] CD40 exposure of thymic epithelial cells in the presence of IFN-γ and IL-1 induced GM-CSF release.[246] Signals through CD40 upregulate the expression of LFA-1, B7 ligands, ICAM-1, and CD23 with involvement in both homotypic and heterotypic cell adhesion and costimulation.[113,115,116,118-120,253-255] In addition, primary PB monocytes express low amounts of CD40 and cytokines, such as GM-CSF, IL-3, or IFN-γ, upregulate this CD40 surface expression.[256] CD40L induces/enhances cytokine secretion of PB monocytes (eg, IL-6, IL-8, and TNF) and potent tumoricidal activity of monocytes.[256] Recently, it was shown that CD40L also costimulates T-cell proliferation and enhances expression of CD25 and CD40L itself.[104] Furthermore, CD40L can transduce signals by its own.[82] Taken together, CD40L may play a complex role in the immune response by functionally interacting with CD40 expressed on the surface of B cells, monocytes, T cells, and some epithelial cells.

A series of HD-derived cell lines (exception the myelomonocytic HD-MyZ cell line) express at the mRNA and protein level not only CD30, but also CD40.[122,196,257,258] On the other hand, these cell lines are negative for CD30L and CD40L mRNA and protein expression.[122,258] Expression of CD40 by H-RS cells has been initially described as an indication for a follicular dendritic cell origin of H-RS cells.[259] Recently, three studies have been reported on high level CD40 expression of primary H-RS cells.[257,258,260] A total number of 156 HD cases have been investigated and primary H-RS cells in 145 HD cases express CD40 (95% of cases positive). HD-involved tissues with all four histologic subtypes expressed abundant amounts of CD40 by H-RS cells independent of CD30 expression and irrespective of their antigenic immunophenotype. Further studies have to investigate the relationship between the expression of cytokines (eg, IL-4 and IFN-γ) and/or EBV proteins (eg, LMP-1) known to upregulate CD40 expression of H-RS cells and the deregulated, strong CD40 expression of H-RS cells. Primary H-RS cells did not express CD40L, but scattered lymphoid cells in disease-involved areas of HD were CD40L$^+$.[258] It is of interest that at least in part the in vitro rosetting of CD40L$^+$ (activated), CD4$^+$ T cells to cultured CD40$^+$ H-RS cells is mediated through the CD40/CD40L adhesion pathway.[257]

Table 5. CD30L and CD40L Share Common Biologic Activities on Cultured H-RS Cells

Function	CD30L	CD40L
Mitogenic activity	+	–
Enhanced clonogenic colony formation	+	+
Induction of cytokine secretion	+	+
Induction of ICAM-1 surface expression	+	+
Increased soluble ICAM-1 concentration	+	+
Aggregation/adhesion	+	+
Upregulation of costimulatory molecules (eg, B7 ligands)	+	+
Downmodulation of CD30 surface expression	+	+
Shedding of CD30	+	+
Shedding of CD40	+	+(?)

Functional analysis of the CD40$^+$ cultured H-RS cells showed a 50% increase of colony formation using a soft agar system,[257] but CD40L had no mitogenic activity on these CD40$^+$ HD-derived cell lines.[258] Recombinant CD40L induced IL-8 secretion and enhanced IL-6, TNF, and LTα from cultured H-RS cell lines.[258] In addition, CD40L enhanced the expression of activation and adhesion molecules, such as ICAM-1, B7-1, and B7-2, all of which are overexpressed on primary H-RS cells.[117,258,261-266] Furthermore, CD40L induced a 40% to 60% reduction of the expression of the HD-associated CD30 antigen with an increase of sCD30 levels.[258]

Taken together, CD30L and CD40L share pleiotropic biologic activities on H-RS cells such as induction/elevation of cytokine secretion and adhesion/activation surface molecule expression (Table 5 and Figs 4 and 6). The CD30-CD30L and CD40-CD40L interactions might be critical elements in the unbalanced cytokine network and cell contact-dependent activation cascade typical for HD.[64]

CD40 EXPRESSION AND MALIGNANT B-CELL NEOPLASIAS

CD40 is found on B cells at most stages of differentiation (with the exception of plasma cells), malignant B cells such as lymphomas and leukemias, and virally transformed B

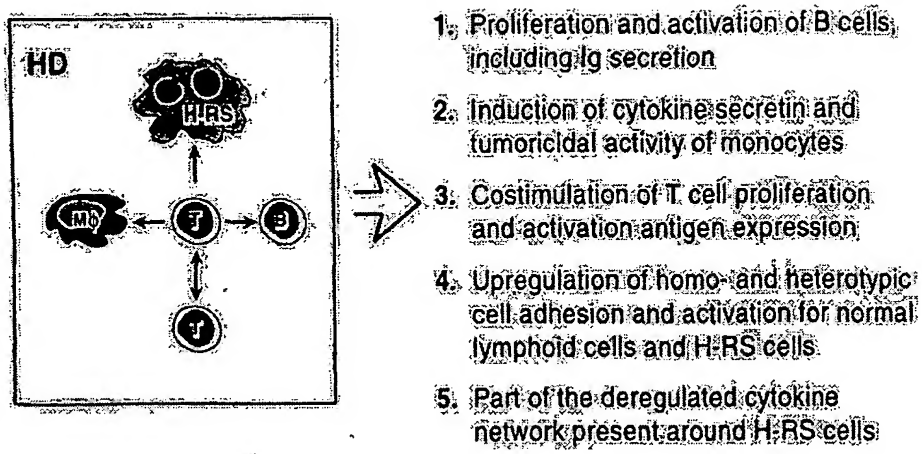

Fig 6. CD40 expression of H-RS cells. In addition to B cells, T cells, and monocytes, most H-RS cells express high levels of CD40 on their surface. CD40L expression is absent from H-RS cells, but is found with higher frequency on some surrounding bystander cells. CD40L might be involved in B-cell activation, costimulation of T cells, and stimulation of monocytes/macrophages, as well as in part of the typical features of HD, such as cellular adhesion and deregulated cytokine secretion.

cells.[26,162,244-246,253,256,267,268] In general, CD40 surface expression is upregulated after activation, but downregulated on differentiation to plasma cells.[162] Antigen-specific activation of B cells requires a two-step signaling pathway with initial antigen binding, processing, and presentation with MHC class II on B cells, followed by recognition of the antigen by helper T cells with activation and expression of costimulatory signals for B cells (T-cell–dependent B-cell help).[106] Collaboration between antigen-presenting B cells and activated T cells is mediated both by soluble proteins (cytokines) and cell-cell contact-dependent membrane-bound (receptor-ligand for cytokines or activation antigens) interaction.[107] A combination of these signals directs a B-cell response with proliferation, antibody production, and isotype switching.

CD40 expression on B cells is of crucial importance for B-cell function.[269] Ligation of CD40 with MoAb induced proliferation of anti-IgM cross-linked or IL-4–stimulated B cells[244,253,270-272]; secretion of IgE, IgG, or IgM in the presence of various cytokines[255,273-276]; rescue of germinal center centrocytes from apoptosis[251,277]; activation of homotypic and heterotypic adhesion[113,115,253-255]; and induction of bcl-2 expression.[277] CD40 expression on B cells is enhanced by IL-4, IgM, CD20 MoAb, PMA, or IFN-γ.[15,26,270] A soluble form of CD40 has been detected in the supernatant of mitogen-activated primary B cells and EBV-transformed B-cell lines.[102]

The cloned CD40L is mainly expressed on activated $CD4^+$ T cells.[26,40,248] Induction of CD40L expression on activated T cells is rapid (maximum, 8 to 10 hours) and is tightly regulated (baseline level after 24 hours of activation).[40,248] As predicted, recombinant CD40L stimulates B-cell proliferation in the absence of costimuli and in combination with cytokines (eg, IL-4 for IgE and IgG_4; IL-2 and IL-10 for IgA, IgG_{1-3}, and IgM) stimulates secretion and/or isotype switching of B cells.[65,249] TGF-β inhibits CD40L-mediated secretion of Igs.[250]

CD40L gene defects have been identified as the causative factor for the inherited condition of a severe immunodeficiency known as X-linked hyper-IgM syndrome, characterized by elevated levels of serum IgM and low or nonexistent levels of IgG, IgA, and IgE; generalized failure to form germinal centers; and increased susceptability to opportunistic infection.[39,153,155,278-280] CD40L is a major pathway involved in T-cell–dependent help for B cells. Taken together, these studies confirm the essential role of CD40 for Ig heavy chain switching and the production of all Ig isotypes other than IgM.

Predicted on the pan-B–cell reactivity of CD40 for lymphoid organs,[162,244,245,253,267,268] several studies indicate that most or virtually all B-cell CLLs (80% to 90% of cases) and B-cell NHLs (90% to 100% of cases) express CD40.[162,244,245] In addition, 30% of biphenotypic or B-lineage ALLs and 90% of HCL express CD40.[245] Notably, the CD40 antigen is restricted to the B-lineage lymphoid cells, because none of the T-lineage ALLs, T-lineage CLLs, or T-lineage NHLs (exception ALCLs) stained with CD40 MoAbs.[245] In contrast, one study reported only 3 of 23 NHLs (12 B-cell NHLs and 11 T-cell NHLs) expressed CD40, including 2 B-cell NHLs and 1 T-cell lymphoblastic lymphoma.[260] These conflicting data may be explained by different sensitivities (affinity and/or off-rate) of the CD40 MoAbs used or the detection system for low (normal) level CD40 expression. CLL B cells and B cells from NHLs activated through CD40 show enhanced DNA synthesis after stimulation with B-cell trophic factors.[281] Of 29 $CD30^+$ ALCL cases investigated, 13 expressed CD40 (45% of cases), but CD40 expression was seen for cases with B-cell, T-cell, or null phenotype.[257,258] Large numbers of tumor cells were labeled and displayed moderate to strong staining.[257,258] These findings further support a pathologic association between $CD30^+$ ALCLs and HD, as suggested by the unique high-level expression of CD30, HD-lectin, IL-9, and c-kit for these two lymphoma entities.[178,282-284] The presence of the ALCL-associated translocation t:2;5 with rearrangement of the NPM gene in at least some HD cases further indicates a biologic relationship between ALCL and some HD cases, but the relationship for a common pathogenesis needs to be identified.[240-242]

Analysis of CD40L presence in vivo confirms restricted expression to small mononuclear cells in lymphoid tissue but not other tissues, such as muscle, brain, kidney, intestine, ovary, uterus, testes, skin, lung, or liver.[285] CD40L expres-

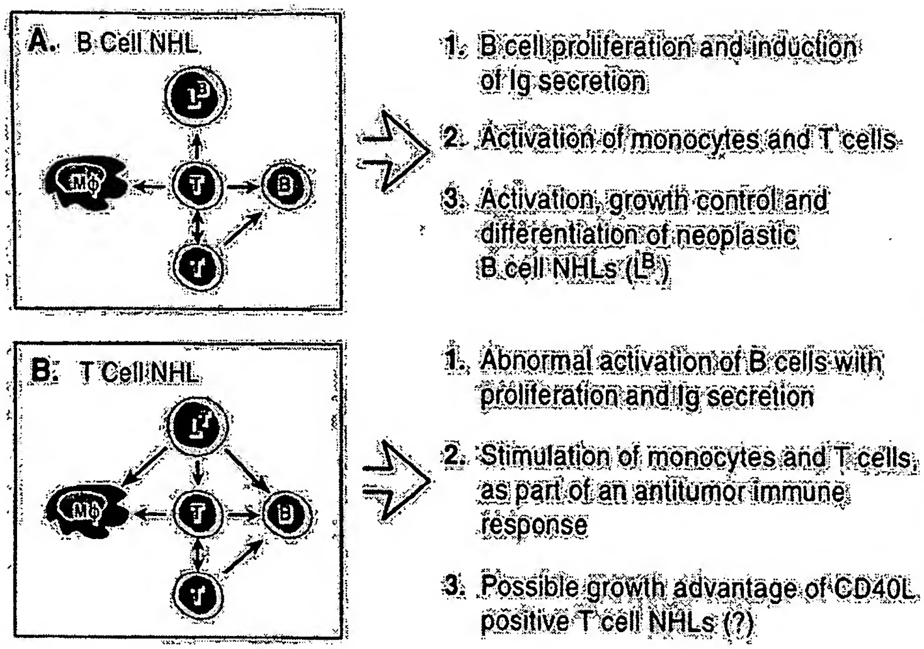

Fig 7. Schematic presentation of CD40-CD40L interaction for different NHLs. CD40 expression is found not only on B cells at most stages of differentiation and virally transformed B cells but also on most malignant B cells (L^B). (A) For $CD40^+$ B-cell NHLs, CD40L expressed by activated surrounding T cells might stimulate B cells, T cells, and monocytes/macrophages and, in addition, may also be involved in activation, growth control, and differentiation of L^B cells. On the other hand (B), $CD40L^+$ T-cell NHLs (L^T) might interact with surrounding $CD40^+$ immune cells and supporting cellular and humoral immune responses.

sion was preferentially localized in the mantle zone and germinal center light zone of secondary follicles of all peripheral lymphoid tissues and also the T-cell–rich periarteriolar areas in the spleen.[285] In vivo, CD40L-expressing cells are mainly $CD4^+$ T cells.[285] A panel of T-cell NHLs and leukemias (87 cases) was investigated for localization of CD40L expression.[286] Immunohistochemical staining showed that 21 of the 87 cases (24%) expressed CD40L with membrane and/or cytoplasmic immunoreactivity of a majority of neoplastic cells. In addition, the unexpected constitutive expression of CD40L by the neoplastic T cells was found mainly for $CD4^+$ tumors with a mature T-cell phenotype. The association of constitutive CD40L expression for some T-cell tumors with clinical and immunologic presentation such as hypergammaglobulinemia, monoclonal gammapathies, immune complex disease, or autoantibody syndromes needs to be established for the abnormal activation of B cells through malignant T cells. CD40L might provide a growth advantage for a subgroup of T-cell neoplasias.

An activation-induced growth arrest has been observed for several B- and T-cell malignancies through either antigen receptor or costimulatory receptors with antitumor effects when exposed to stimuli that lead to activation of the normal counterparts.[287-290] It is of great interest that anti-CD40 and soluble CD40 ligand significantly inhibited (40% to 60%) the in vitro proliferation of a series of B-cell lymphoma cell lines.[291,292] In addition, an antitumor effect of anti-CD40 treatment was observed in SCID mice bearing the human B-cell lymphoma lines with increased survival and inhibition of tumor growth.[291] Furthermore, anti-CD40 MoAb prevents human B-cell lymphomagenesis in the hu PBL-SCID mouse, but also promotes human B-cell engraftment in vivo.[293] CD40L may be of significant clinical use in new treatment modalities of $CD40^+$ NHLs with or without conjugation to immunotoxins or radioisotopes.

Taken together, CD40 expression on malignant B cells seems to be a frequent finding and a potential candidate for alternative treatment approaches, such as tumor targeting (Fig 7). Expression and the pathophysiologic role of CD40L for NHLs requires further functional analysis in vitro and in vivo (Fig 7).

ROLE OF FAS/FASL SYSTEM FOR LYMPHOMAS

Cell survival and function is controlled by proliferation and differentiation (positive selection) but also by cell death (negative selection).[294,295] The death of cells occurs by either programmed cell death with apoptosis or necrosis.[296,297] Apoptosis can be morphologically and biochemically distinguished from necrosis. In most cases, apoptosis is associated with condensation and segmentation of nuclei, loss of plasma membran microvilli, and degradation of chromosomal DNA into 200-bp nucleosome fragments.[295,297]

FAS and APO-1 antigens were identified by MoAbs with cytolytic activity against certain human cells and subsequently cDNA cloning.[18,19,134,135] FAS and APO-1 antigen are identical molecules and have been assigned to cluster CD95.[18,19,298] The CD95 antigen acts as a cell-surface receptor that is involved in apoptosis, including T- and B-cell deletion for the immune system.[47,126-136] In vivo, CD95 MoAbs induce rapid tumor regression.[134,299]

CD95 expression is found on myeloid cells, fibroblasts, and activated lymphocytes, as well as on various lymphoma and leukemia cells, but is not always associated with cell death.[18,123,124,128,134,135,148,298,300,301] In addition, lymphoblastoid cells transformed with HTLV-1 and -2, HIV, or EBV also highly express functional CD95 antigen.[302-304] CD95 is also expressed in tissues such as liver, heart, lung, and ovary.[298] Detailed analyses have shown that the CD95 antigen is weakly expressed on the surface of most malignant B cells isolated from CLLs, but is upregulated by stimulation with

Staphylococcus aureus Cowan I (SAC) or IL-2 and showed CD95-mediated apoptosis.[305] In contrast, HCL B cells expressed CD95 at moderate levels.[305] The induction of CD95-mediated apoptosis was correlated in some instances with bcl-2 downregulation.[305] The bcl-2 expression is correlated with inhibition of apoptosis[306] and deregulation of bcl-2 expression might be part of the pathogenesis of lymphomas (eg, follicular lymphomas) and leukemias (eg, B-cell CLL).[307] In addition, mediastinal B-cell lymphomas coexpress, depending on their differentiation stage, CD95 and CD54.[308] Interestingly, B cells from one CLL cases did not show bcl-2 downregulation after stimulation with SAC and IL-2, and CD95 MoAb induced a proliferative signal.[305] Further, one case of B-cell lymphoma stimulated with IL-14 also showed significant growth enhancement with CD95 MoAb treatment.[309] Similarly, fresh PBTs showed a costimulatory response in the presence of CD95 MoAbs or sCD95L, but chronic activated PBTs or T-cell clones underwent apoptosis.[123,128,300,302,310-312]

CD95 expression of Burkitt's lymphoma (BL) cell lines is associated with a lymphoblastoid phenotype.[304] EBV^- and EBV^+ BL cell lines with type I phenotype ($CD10^+$, $CD21^-$, $CD23^-$, $CD30^-$, $CD39^-$, $CD70^-$, $CD77^+$) corresponding to primary BL tumor cells have no detectable CD95 surface expression. Accordingly, primary BL tumor cells (3 cases) were also $CD95^-$. In contrast, EBV^+ BL cell lines with type III lymphoblastoid phenotype ($CD10^-$, $CD21^+$, $CD23^+$, $CD30^+$, $CD39^+$, $CD70^+$, $CD77^-$) as well as normal lymphoblastoid B-cell lines expressed the CD95 antigen at high density, but 6 of 7 $CD95^+$ BL cell lines were not sensitive to CD95-mediated killing. In addition, only one of eight B-cell NHLs and one of two T-cell NHLs expressed low levels of CD95, which was upregulated by IL-14.[309] The expression of the CD95 antigen has been reported for the malignant cells of 83% (10 of 12 patients) of follicular lymphoma cases and 56% (18 of 32 patients) of diffuse lymphoma cases.[313] Subgrouping into different histiologic subgroups showed similar distribution for all categories.[313] In addition, all adult T-cell leukemia cases (n = 12) were $CD95^+$ and underwent apoptosis by adding CD95 MoAbs.[314] The detailed functional role of CD95 for cell survival and tumor growth is presently not well understood. Recently, the cognate for CD95 (CD95L/FASL) has been cloned and characterized as a 31-kD type II transmembrane protein with 25% to 30% homology to other members of the TNF ligand superfamily.[47] Recombinant CD95L exists also in a soluble form, with similar biologic activities seen for the CD95 MoAbs or membrane-bound CD95L.[47] The physiologic presence and role of the sCD95L remain to be determined.

CD95 and CD95L expression and function has not been well investigated for HD, but the HD-derived cell lines HDLM-2, KM-H2, L-428, and L-540 express CD95 and show apoptosis after treatment with CD95 MoAbs or soluble CD95L (H.J.G., manuscript in preparation).

In general, CD95 shares a dual role with the ability to mediate stimulatory or inhibitory/cytotoxic signals depending on the target cells or activation stage. The CD95/CD95L-mediated T-cell cytotoxicity could play a major role in controlling the immune response of peripheral lymphocytes and might be involved in T-cell tolerance.[315,316] In general, the cloning of the CD95L will further improve our understanding of the mechanisms of apoptosis and the functional relevance of CD95(FAS)-CD95L(FASL) interaction for malignancies, particular lymphoid tumors, such as a variety of lymphomas and leukemias with B- and T-cell phenotype.

4-1BB/4-1BBL INTERACTION AND THE PATHOGENESIS OF LYMPHOMAS

4-1BB was identified and cloned from activated T cells (activation induced cDNA clone).[10,16,44,317,318] The 33-kD 4-1BB molecule is expressed on activated T cells ($CD4^+$ and $CD8^+$) and thymocytes.[16,317,319] 4-1BB antibodies have costimulatory activity for T-cell proliferation.[319] Initially, it was reported that extracellular matrix proteins bind 4-1BB, but the functional relevance remains unclear.[320] Subsequently, murine and human 4-1BB ligands were identified and expression cloned.[43,44] Expression of 4-1BBL was found for activated T cells, stromal cells, activated macrophages, EBV-transformed B cells, some tumor and leukemia cell lines, and a variety of tissues such as brain, placenta, lung, skeletal muscle, and kidney.[43,44,84] 4-1BBL costimulates T-cell and thymocyte proliferation, but other biologic activities for the immune system and hematopoesis that are indicated by the wide distribution pattern of 4-1BB and 4-1BBL need to be identified. It is of additional interest that signals through 4-1BB enhance activation-induced cell death (AICD) of T cells.[44] 4-1BBL is able to function as a signal transducing molecule.[84] 4-1BB and 4-1BBL are expressed on activated T cells and could play an autocrine regulatory role in T-cell interaction.[43,44,84] Primary lymphomas have not been analyzed for 4-1BB and 4-1BBL expression, but a series of HD-derived cell lines express, in addition to the TNFRs, CD30, CD40, and FAS also 4-1BB (H.J.G., manuscript in preparation). The functional and pathologic relevance needs to be examined.

THE OX40 MOLECULE AND HD

The OX40 molecule was originally described as a cell surface antigen on activated rat T cells[321]; subsequently, the genes encoding rat, mouse, and human OX40 have been cloned.[45,46,322-324] Expression of OX40 was reported initially to be restricted to activated $CD4^+$ T cells.[322,323] The human OX40 molecule is identical to the ACT35 antigen, described as strictly activation-associated antigen.[162,324] No expression was found for resting peripheral blood lymphocytes, peripheral blood B cells, and thymocytes. In lymphoid tissue, huOX40 expression was seen for scattered cells in the interfollicular zone, the follicular mantle zone, and the germinal centers.[162] Tissue macrophages were more weakly positive. For HD, only a few cases showed OX40 expression of H-RS cells, but T cells surrounding H-RS cells in a rosette fashion were strongly positive.[162] Recently, murine and human OX40L have been cloned from the murine lymphoma cell line S49.1 or the activated B-lymphoblastoid cell line MSAB, respectively, and the human homologue identified to be gp34, a protein expressed on HTLV-1–infected human

leukemic T cells.[45,46,325,326] OX40L expression is, as the OX40 receptor, selectively induced on activated $CD4^+$ and $CD8^+$ T cells, but not on B cells.[45] The OX40L is also expressed on HTLV-1–transformed cell lines, stimulated B-lymphoblastoid cell lines, and THP-1 cells.[46] As predicted, human OX40L costimulates T-cell proliferation and cytokine production (eg, IL-2 and IL-4) as part of the regulatory cascade for immune resonses.[45,46] A possible correlation between the OX40/OX40L system and virally induced pathogenesis needs further evaluation. Detailed expression and functional analyses have to be performed to understand the role of OX40-OX40L for the pathogenesis and/or tumorigenesis of lymphomas, particularly in the context of viral transformation (eg, EBV, HTLV, and HIV).

TNF AND LT EXPRESSION IN HD WITH UNCLEAR BIOLOGIC RELEVANCE

TNF was originally defined by its antitumor activity but is also a major mediator of inflammation and cellular immune response.[8] TNF was found to be cytotoxic to a number of transformed cell lines in vitro.[327] TNF induces cachexia in LPS-treated mice with profound effects on general cellular metabolism and development of weight loss, fever, acute phase reaction, infection, or neoplasia.[328] TNF enhances the proliferation of T cells, modulates T-cell receptor expression, enhances NK cell activity, and regulates human B-cell function. TNF also has marked effects on neutrophils, eosinophil recruitment, monocyte/macrophage activation, fibroblast growth stimulation, and endothelial cell/leukocyte interactions.[329] TNF is produced by many cell types, including monocytes/macrophages, lymphocytes, and fibroblasts.[8] Activated macrophages have the highest TNF production.[328]

LT is a cytokine structurally related to TNF with approximately 50% sequence homology, the same chromosomal localization, and trimer structure.[8] LT is synthesized primarily by T cells, although some EBV-transformed B-cell lines and tonsil B cells produce it.[8] LT and TNF have similar but not identical inflammatory and immunomodulatory activities.[8] LT is often less potent than TNF. Initially, LT (TNF-β) was cloned as a soluble cytokine.[330] Surface LT does not result from the presence of the transmembrane region but, rather, was found associated with a 33-kD integral membrane glycoprotein.[75,76] The cloned gene encoding this second protein in the surface LT complex was found to be a new member of the TNF ligand superfamily.[35] Recently, the LT complex units have been renamed as LTα (TNF-β/LT) and LTβ (p33), being synthesized in soluble form as a LTα homotrimer or as a membrane-anchored heterotrimeric complex composed of LTα and LTβ units (eg, $\beta_2\alpha_1$).[35] Generic ligand-receptor interaction analysis predicts that the heterotrimeric LTβ complex would produce a functionally inactive receptor-binding protein.[35,77] The immunologic function and biologic role of LTβ is presently not well understood.

Receptors for TNF and LT are expressed at low levels on most tissues and various cell types.[8,331] Three distinct receptors have been shown to bind TNF, LTα, and LTβ.[9-11,77] The p60 TNFR type I and p80 TNFR type II cDNAs encode distinct proteins with 20% homology in the ligand-binding domain (extracellular domain); both receptors bind TNF and LTα with similar affinities.[9-11] The heterotrimer LTβ binds to the recently identified TNFR-RP surface protein (TNFR-III).[12,77] Expression of TNFRs on human PBT cells is activation dependent.[332] TNFR expression is upregulated by agents such as IL-2, IFN-α, cAMP, and different hormones but is downregulated by IL-1, PMA, glucocorticoids, and LPS.[8] Soluble forms of TNFR-I and TNFR-II have been identified in the serum of normal persons and tumor patients.[91,94,95,97] It is of particularly interest that several viral ORFs such as SFV-T2, MYX-T2, G4R, crmB, Va53, and SalF19R, encode soluble homologues of the TNFR proteins with a possible role in viral host response.[10,20-22,333,334] These viral ORFs show a novel mechanism of viral subversion of the host immune response.[67]

Expression of TNF and LTα protein and mRNA has been reported for a series of HD-derived cell lines.[196,258,335-338] Similarly, TNF expression of H-RS cells was shown in primary tissue from HD patients.[175,265,336,337,339,340] Using immunohistochemistry and/or in situ hybridization, 56 of 104 cases investigated (54% positive cases) showed strong cytoplasmic TNF signals for H-RS cells. In addition, immunoreactivity for TNF was observed for some macrophages. Furthermore, abundant LTα expression was found on primary H-RS cells for 47 of 59 HD cases analyzed (80% of cases positive).[175,339] In comparison with TNF, the LTα-specific mRNA signals were usually of higher intensity and present in larger proportions of H-RS cells.[175] TNF and LTα gene transcripts were also seen in some lymphoid cells.

Most HD-derived cell lines express the p60 and p80 TNFRs on their surface or at the mRNA level.[196,335] The presence of TNFR-RP protein on cultured or primary H-RS cells has not been investigated. Limited data are available for the TNFR expression in HD cases. One study reported the expression of p60 TNFR in 1 of 4 (25%) and p80 TNFR in 2 of 4 (50%) HD cases analyzed using immunohistochemistry.[341] Further detailed expression studies for primary HD material are required to fully understand the involvement of TNF and LTα for the pathogenesis of HD, including growth control for H-RS cells.

These different studies indicate that TNF and LTα expression of the malignant H-RS cells in HD-involved tissue is a frequent finding and could have a central functional relevance for oncogenesis and pathogenesis of HD (Fig 8). High-level expression of LTα for cultured and primary H-RS cells is a striking feature for HD, with unknown biologic correlation.[64] Primary and cultured H-RS cells express not only TNF and LTα but also the TNFRs, suggesting a possible autocrine growth loop for H-RS cells (Fig 8). TNF and LTα lack mitogenic activity on cultured H-RS cells and a presumed autocrine growth loop, based on coexpression of corresponding ligands and receptors, has so far not been shown.[342] It is of interest, that recombinant CD30L and CD40L induce the secretion of TNF and LTα.[117,258]

A number of typical pathologic and clinical features of HD are consistent with characteristics of a tumor of cytokine-producing cells, including occurence of B symptoms, sclerosis, eosinophilia, acute-phase responses, T-cell rosetting and activation, impaired immune responses, and generalized itching.[54] Furthermore, cytokines produced by H-RS cells

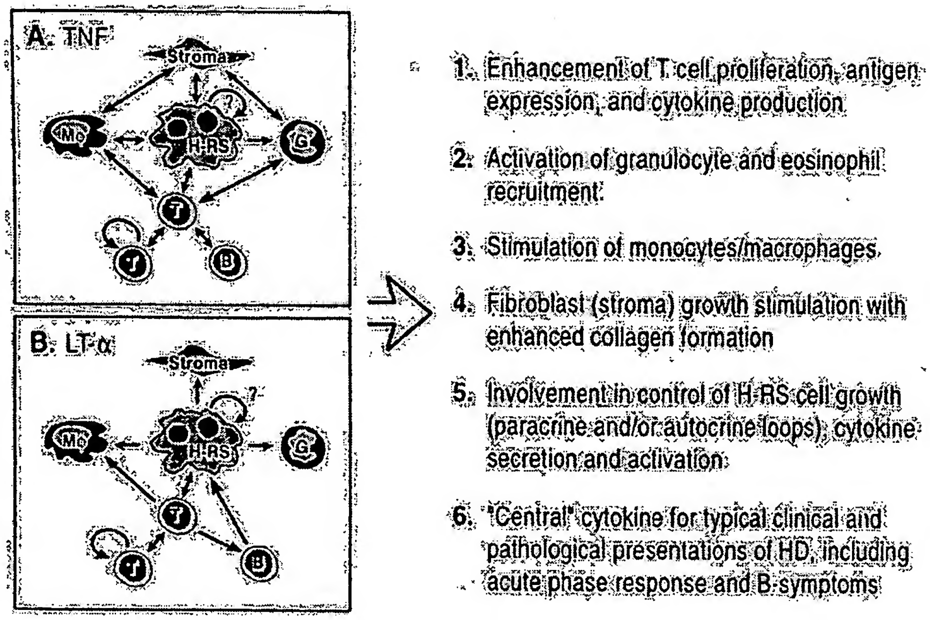

Fig 8. TNF and LTα involvement in the interaction between H-RS cells and surrounding bystander cells. TNF (A) is produced by many cell types, such as monocytes/macrophages, lymphocytes, and stroma cells. Expression of TNF and TNFRs is a common finding for H-RS cells. TNF involvement in paracrine and/or autocrine growth stimulation of H-RS cells remains unclear. TNF could also be critical for accumulation/activation of bystander cells and one mediator for systemic B symptoms. Similarly, LTα (B) is frequently overexpressed by H-RS cells in HD. In contrast to TNF, LTα expression is mainly restricted to lymphoid cells. The functional role of LTα for HD is presently unclear.

might interact with surrounding bystander cells, particularly T cells; conversely, H-RS cells might respond to cytokines produced by surrounding normal reactive bystander cells. TNF and LTα are part of these deregulated cytokine network involved in HD. TNF and LTα could be involved in causing fever, weight loss, and night sweats, as cytokines involved in the development of the constitutional B symptoms.[328] TNF is also mitogenic for fibroblasts and induces collagen synthesis with a potential role in formation of sclerosis.[329] Elevation of, eg, fibrinogen or prostaglandin serum levels, frequently seen in HD, could be associated with TNF secretion.[54,343] It is of interest that HD patients have elevated TNF serum levels (47 of 76 HD patients [62%]); the extent of increase correlated with disease stage and the presence or absence of B symptoms.[344] Similarly, the mean serum levels of the soluble p60 TNFR were significantly higher in HD patients than in healthy controls.[344] The degree of increase correlated with TNF serum levels, disease stage, and disease activity (presence of B symptoms). Furthermore, increased soluble p60 TNFR serum levels of HD patients in remission could be involved in the cellular immune defect characteristic for HD patients.[345,346] The elevated soluble TNFR concentration might support the escape of H-RS cells in the predisposing immunocompromised host from the tumor-suppressive effects of TNF and LTα and also cellular antitumor immune response by blocking appropriate T-cell activation and function. In addition, systemic TNF might be causative with other immune modulators, such as IL-1 and IL-6, in development of B symptoms and/or metabolic wasting (Fig 8).

TNF AND TNFR INTERACTION FOR NHLS

Immunohistochemical studies of nonmalignant, reactive cells showed that TNF-positive cells were rarely detected in lymph nodes with activation of the B-cell compartment but were frequently detected in sections from patients with diffuse or mixed lymphadenitis with expansion of the T-cell–dependent areas.[265,347,348] As presumed, strong TNF signals were associated with the presence of macrophages. The analysis of 20 NHL cases showed only 4 of 16 B-cell NHL cases showed weak scattered TNF-positive cells.[265] In the four T-cell NHLs, TNF was not detectable on the neoplastic lymphoma cells but was detectable on macrophages in T-cell paracortical areas.[265] In contrast to the high frequency of TNF and, particularly, the LTα expression of the neoplastic H-RS cells in HD, the neoplastic lymphoma cells of NHLs seems only rarely to produce TNF and LTα as an indicator of their malignant transformation. Similarly, RNA prepared from nonmalignant, reactive lymph nodes confirmed low expression of TNF and LTα.[339] In contrast, moderate to abundant levels of TNF mRNA were detected in 12 of 35 NHL specimens (33% of cases positive; 9 low-grade and 3 high-grade NHLs).[339] In contrast, in situ hybridization with IL-6, TNF, and LTα probes of eight lymphoplasmacytic lymphomas lacked IL-6, TNF, and LTα expression.[175] Variable amounts of increased LTα mRNA were detected in 19 of 35 NHL specimens (54% of cases positive).[339] Ten of the NHL cases coexpressed TNF and LTα mRNA.[339] Interestingly, 8 of 12 lymphoma patients (67% of cases) with the presence of systemic B symptoms had high TNF mRNA levels and 11 of these 12 lymphoma cases (92% of cases) had also abundant LTα mRNA expression.[339] High TNF and LTα mRNA expression correlated significantly with the presence of systemic B symptoms. Similarly, murine and human NHL cell lines express either constitutively TNF and LTα mRNA and protein or can be induced.[8,349] Further detailed studies are needed to confirm these initial results and to demonstrate the localization of TNF/LTα-expressing cells.

So far, only one study analyzed p60 and p80 TNFR expression systematically by using immunohistochemistry for normal tissues and NHL specimens.[341] For the normal

lymphoid tissue sections, p60 TNFR expression was restricted to dentritic reticulum cells of germinal centers, but p80 TNFR was found on a major cell population of interdigitating cells and activated lymphocytes in the interfollicular T-cell areas. The p60 and p80 TNFR expression sites are different and could allow different biologic function of TNF and LTα with either paracrine or autocrine signaling pathways. In reactive lymph nodes, the number of p80 TNFR-positive cells was increased in the T-cell areas.[341] The analysis of a limited number of B- and T-cell NHL sections (n = 30) showed that mainly lymphoma cells with a high-grade malignant phenotype (6 of 14 B-cell NHLs and 6 of 8 T-cell NHLs) expressed p80 but lacked p60 TNFR expression.[341]

TNF and LTα have important roles in normal B-cell activation, growth, and differentiation.[108,109,350] TNF is capable of inducing proliferation of the TNFR-positive neoplastic B cells from CLL patients.[351] Very recently, it was shown that lymphoblastoid B-cell lines and BL cell lines with a lymphoblastoid phenotype ($CD10^-$, $CD77^-$, $CD23^+$, $CD40^+$) use LTα as an autocrine growth factor and act mainly through the p60 TNFR.[352] Overall, the expression and functional relevance of the cytokines TNF and LTα for NHLs remain unclear. Malignant B cells from CLL patients use TNF as a growth factor, but similar data are presently not reported for NHLs. Further studies have to confirm the expression not only of TNF and LTα but also of the two TNFRs for different NHL entities as well as the potential biologic relevance for growth control, differentiation, and activation of NHL cells.

SUMMARY

The TNF receptor superfamily members are all type I membrane glycoproteins with typical homology in the extracellular domain of variable numbers of cysteine-rich repeats (overall homologies, 25% to 30%). In contrast, the TNF ligand superfamily members (with the exception of LTα) are type II membrane glycoproteins with homology to TNF in the extracellular domain (overall homologies, 20%). TNF and LTα are trimeric proteins and are composed of β-strands forming a β-jellyroll. The homology of the β-strand regions for the TNF ligand superfamily members suggest a similar β-sandwich structure and possible trimeric or multimeric complex formation for most or all members. A genetic linkage, as evidence for evolutionary relatedness, is found by chromosomal cluster of TNFR p80, CD30, 4-1BB, and OX40 for 1p36; TNFR p60, TNFR-RP, and CD27 for 12p13; TNF, LTα, and LTβ for 6 (MHC locus); CD27L and 4-1BBL for 19p13; and FASL and OX40L for 1q25.

Of the TNF ligand superfamily, TNF, LTα, and LTβ and their receptors (TNFR p60, TNFR p80, and TNFR-RP) interact in a complex fashion of cross-binding. However, the other family members presently have a one ligand/one receptor binding principle (CD27/CD27L, CD30/CD30L, CD40/CD40L, 4-1BB/4-1BBL, OX40/gp34, and FAS/FASL). In general, the members of the TNF ligand superfamily mediate interaction between different hematopoietic cells, such as T cell/B cell, T cell/monocyte, and T cell/T cell. Signals can be transduced not only through the receptors but also through at least some of the ligands. The transduced signals can be stimulatory or inhibitory depending on the target cell or the activation state. Taken together, TNF superfamily ligands show for the immune response an involvement in the induction of cytokine secretion and the upregulation of adhesion molecules, activation antigens, and costimulatory proteins, all known to amplify stimulatory and regulatory signals. On the other hand, differences in the distribution, kinetics of induction, and requirements for induction support a defined role for each of the ligands for T-cell–mediated immune responses. The shedding of members of the TNF receptor superfamily could limit the signals mediated by the corresponding ligands as a functional regulatory mechanism. Induction of cytotoxic cell death, observed for TNF, LTα, CD30L, CD95L, and 4-1BBL, is another common functional feature of this cytokine family. Further studies have to identify unique versus redundant biologic and physiologic functions for each of the TNF superfamily ligands.

Primary H-RS cells can express TNF, LTα, and CD27L but not CD30L and CD40L, in addition to IL-1α, IL-5, IL-6, IL-9, and M-CSF. In addition, H-RS cells express high copy numbers of several cytokine receptors such as IL-2R (p55, p75, and p64 subunits), IL-6R, M-CSFR (c-fms), SCFR (c-kit), CD30, CD40, and TNFRs.[64] Cytokines produced by H-RS cells might support the growth of tumor cells (autocrine growth loop) and/or interact with surrounding reactive bystander cells, particularly T cells. Conversely, H-RS cells might respond to cytokines produced by surrounding reactive normal cells (paracrine growth loop). The different interactions between H-RS cells and surrounding normal, reactive bystander cells, such as lymphocytes, plasma cells, histiocytes, neutrophils, eosinophils, and stromal cells, is characteristic for HD. The expression and biologic effects of a panel of cytokines and their counterpart receptors seem to be involved in the pathobiologic interaction between H-RS cells and particularly lymphocytes, mainly $CD4^+$ T cells. Detailed analyses have to verify the predicted biologic activities of TNF, LTα, CD27L, CD30L, CD40L, 4-1BBL, gp34/OX40L, and FASL for the H-RS cell/T-cell interactions with impact on tumor growth and pathogenesis of HD. Cytokines and cytokine receptors, including TNF/TNFRs, CD30/CD30L, and CD40/CD40L, are clearly critical elements in the pathology of HD and are part of the deregulated network of interactive signals between H-RS cells and surrounding bystander cells with membrane-associated and cytokine-mediated events. HD is a tumor of cytokine-producing cells that is causative for several characteristic clinical and pathologic presentation of HD.

The functional role of cytokines for the pathogenesis of NHLs is presently unclear. Malignant NHL cells express, depending on their immunophenotype, several TNF receptor and ligand superfamily members. B-cell NHLs are frequently CD27/CD27L, CD30 or CD30L, CD40, and TNFRs/TNF positive, but T-cell NHLs have expression of CD30, CD40L, and TNFRs/TNF. Further functional analysis will increase our understanding of the involvement of TNF superfamily ligands in the pathogenesis of NHLs.

Several TNFR superfamily members could be candidates for novel treatment protocols. Recombinant CD30L and CD40L could be by itself antitumorigenic for $CD30^+$ ALCLs

and $CD40^+$ B-cell NHLs, respectively. Furthermore, CD30 and CD40 might be used for tumor targeting after conjugation with radioisotypes or cytostatic drugs for $CD30^+$ and/ or $CD40^+$ HD and NHLs.

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